1. Export the imaging mass spectrometry (IMS) data using python code (.csv file)
2. Upload to .csv matlab and select region of interest
3. Upload autoflorescence (AF) and H&E image
4. Register IMS to AF image (make sure the IMS and AF images are in the same orientation):
   1. [mp,fp] = cpselect(IMS,AF,'Wait',true)
   2. t\_IMS\_AF = fitgeotrans(mp,fp,'similarity')
   3. Rfixed\_AF = imref2d(size(AF))
   4. IMS\_AF = imwarp(IMS,t\_IMS\_AF,'OutputView',Rfixed\_AF)
   5. imshowpair(IMS\_AF,AF) #check the quality of registration, repeat a-d if not satisfactory
5. register AF to H&E and then register IMS to H&E (make sure the H&E and AF images are in the same orientation):
   1. [mp,fp] = cpselect(AF,HE,'Wait',true)
   2. t\_AF\_HE = fitgeotrans(mp,fp,'similarity')
   3. Rfixed\_HE = imref2d(size(HE))
   4. IMS\_HE = imwarp(IMS\_AF,t\_AF\_HE,'OutputView',Rfixed\_HE)
   5. imshowpair(HE,IMS\_HE) #check the quality of registration, repeat a-d if not satisfactory
6. Expand features of the H&E image to 120:
   1. HE\_AF\_1 = single(HE)
   2. HE\_AF\_l(:,:,4:6) = single(rgb2lab(HE))
   3. HE\_AF\_1(:,:,7:9) = single(rgb2hsv(HE))
   4. HE\_AF\_1(:,:,10:12) = single(rgb2ntsc(HE))
   5. HE\_AF\_1(:,:,13:15) = single(rgb2ycbcr(HE))
   6. HE\_AF\_1 = reshape(HE\_AF\_1,[x\_dimension\*y\_dimension, 15])
   7. [[coeff,score,latent] = pca(**HE\_AF\_1**)](https://www.mathworks.com/help/stats/pca.html#d123e694434)
   8. HE\_AF\_1(:,16:30) = score
   9. HE\_AF\_1(:,31:60) = single(rangefilt(HE\_AF\_1))
   10. HE\_AF\_1(:,61:120) = single(entropyfilt(HE\_AF\_1)
7. Save registered IMS and HE as .csv file for python codes

writematrix(HE\_AF\_1, ‘HE.csv’)

IMS\_HE = reshape(IMS\_HE,[x\_dimension\*y\_dimension,1])

writematrix(IMS\_HE,'IMS.csv')

1. Linear Regression
   1. IMS\_HE = reshape(IMS\_HE,[x\_dimension\*y\_dimension,1])
   2. mdl = fitlm(HE\_AF\_1,IMS\_HE )
   3. predicted\_image = reshape(mdl.Fitted,[x\_dimension, y\_dimension])
2. PLS regression
   1. IMS\_HE = reshape(IMS\_HE,[x\_dimension\*y\_dimension,1])
   2. [[XL,YL,XS,YS,BETA,PCTVAR,MSE,stats] = plsregress(HE\_AF\_1,IMS\_HE,ncomp)](https://www.mathworks.com/help/stats/plsregress.html#d123e729651)
   3. Predicted\_image = [ones((size(HE\_AF\_1,1)),1) HE\_AF\_1]\*BETA
   4. Predicted\_image = reshape(Predicted\_image,[x\_dimension, y\_dimension])